

BSC Nomination Review

Meeting of the National Toxicology Program Board of Scientific Counselors

National Institute of Environmental Health Sciences
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NTP Study Nomination: Artificial butter flavoring and constituents diacetyl and acetoin

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Artificial butter flavoring (ABF) is used widely, from food products used in home cooking to manufactured food products, including popcorn as well as other foods; ABF or its components, including diacetyl and acetoin, are also used in the production of flavorings, the manufacturing of dairy products, food preparation involving cooking with soybean and other seed oils; composting biowaste, charcoal production, pig farming, industries involving fermentation with selected bacteria, and carpet laying (diacetyl). The most serious problems observed to date have been in the popcorn manufacturing business, especially microwave popcorn. Increasing numbers of these workers are developing obstructive lung disease, presumably from the vapors of ABF (two main ingredients of these vapors are diacetyl and acetoin); these may progress to bronchiolitis obliterans (over a dozen workers diagnosed, and another dozen or so suspected, at 5 plants); at least 3 deaths have been reported in a microwave popcorn manufacturing plant and other workers are candidates for lung transplants. Many other workers had evidence of deteriorating pulmonary function. "...Production area workers had significantly higher rates of shortness of breath on exertion, breathing problems, a combination of respiratory symptoms, unusual fatigue, other systemic symptoms, and rashes or other skin problems... Other industries that have reported incidence of bronchiolitis obliterans...include "a baking industry mixing facility flavoring manufacturing, and a snack food manufacturer. "As cumulative exposure to diacetyl increased, the incidence of airway obstruction and abnormal results on spirometry... increased and the average one second forced expiratory volume decreased." The severity of the health effects associated with ABF and the fact that more than one manufacturer is involved make this a most appropriate nomination as well as an appropriate use of NTP resources.

1. Does the NTP research concept address the needs of the nomination?

The nomination requests long term testing of artificial butter flavoring (ABF), diacetyl, and acetoin for respiratory toxicity and carcinogenesis with inhalation exposure. The intent is to

protect workers from severe and even fatal lung disease or from developing cancer associated with workplace exposure to ABF, its major components diacetyl and acetoin, or compounds with related chemical and physical properties.

The NTP research concept as written summarizes the nomination rationale for examining inhalation toxicity and provides some background information regarding knowledge gaps. The studies on diacetyl conducted by NIEHS/NTP are summarized and include the formation of diacetyl-arginine adducts, acute inhalation and oropharyngeal aspiration toxicity studies in C57Bl/6 mice, and some evidence of systemic inflammation with subcutaneous administration in Balb/c mice. The primary target of ABF and diacetyl in rodents is the nasal cavity whereas toxicity in the bronchioles is associated with human ABF exposure. These differences will complicate extrapolation of dose response data obtained in rodents to decisions regarding human health.

In considering the specific aims of the proposed research program and the proposed studies:

- Communication with NIOSH scientists and coordination of study efforts with NIOSH is essential to avoid duplication of efforts, especially regarding immunotoxicity studies. NIOSH scientists appear to have a number of past and recent publications concerning diacetyl and ABF.
- The research concept is responsive to the nomination in that it proposes studies to assess subchronic inhalation toxicity of ABF, diacetyl and acetoin. The highest priority is to set inhalation exposure limits and protect workers from exposure by the route that is most likely responsible for the toxicity. Assessing subchronic exposure is important because adverse health effects have occurred in occupational settings where diacetyl concentrations are well below levels where acute toxicity occurs in animals. The BSC agreed that because of the severity of the lung disease and the association with ABF exposure, there should be NO DELAY in setting initial exposure limits for ABF. These limits can then be modified as animal data becomes available.
- Differences in ABF formulations may provide information regarding the hazardous component(s) of ABF. How is the ABF formulation for evaluation chosen?
- Studies by Kreiss et al suggest that a high proportion of workers in a plant manufacturing diacetyl develop BO. Thus, this provides a sound rationale to concentrate studies on the diacetyl component of the ABF. However, ABF as a mixture should continue to be evaluated.
- The research concept does not address the nominators request for evaluation of carcinogenicity by the inhalation route. However, this is lower priority than the proposed studies given the known association of ABF exposure and development of fixed airway obstruction. It was noted that the subchronic studies will automatically provide initial information about carcinogenicity.

- Whether aspiration studies would be more relevant to the human exposure should be considered because of the species differences in respiration. The current proposal includes whole body inhalation exposure of rats and mice. The investigators should consider using low dose aspiration studies chronically to determine no affect levels.

It is presumed that the 2 week and 13 week studies will be conducted with the ABF mixture as well as the individual components diacetyl and acetoin.

2. Is the proposed study approach as outlined in the research concept document appropriate in scope given the merit of the nomination? Are there other studies that should be considered for this substance?

The proposed studies are likely the first ones needed to provide data for setting permissible exposure limits. The proposed studies go beyond the scope of the nomination in that they propose the assessment of immunotoxicity. The data to indicate that ABF causes immunotoxicity in humans is not well outlined or apparent in the research concept or the background material. However the animal studies outlined suggest that immunotoxicity is a possibility. It was suggested that a standard immunotoxicity panel of tests might not be as valuable as more targeted hypersensitivity testing of ABF in the LLNA or evaluation of Th1/Th2 cytokines in the lung.

Other studies to be considered might include evaluation of systemic or respiratory toxicity with dermal exposure. Diacetyl alone is a weak sensitizer in the LLNA. However the evidence ruling out dermal exposure in the workers as being insignificant or unimportant in causing toxicity is unclear. Certainly with some sensitizers, dermal sensitization can result in respiratory problems on inhalation challenge. Also the systemic inflammation associated with subcutaneous administration in mice suggests that problems could also be due to the dermal route of exposure.

There should be some discussion of *how* “the aforementioned species differences in target sites [will] be considered when using rodent toxicity data for setting exposure limits for humans.” Some concern was also expressed that because of the limitations of inhalation studies in animals, that such studies will add little new information.

Therefore, continued studies should also concentrate on evaluating actual workplace exposures and their association with lung disease.

3. Does the proposed research program address an important area of biomedical research (e.g. children’s health, genetic susceptibility, specific environmental disease) and/or advance the field of environmental health sciences?

Bronchiolitis obliterans is an important area of biomedical research because many different acute exposures and infectious diseases are associated with this rare, but very serious lung disease. Diagnosis of BO is difficult and treatment for BO is lacking, short of transplantation. Information gained from these studies will be valuable in understanding the lung disease in general and will be applicable to numerous other occupational exposures associated with exposures beyond the flavoring industry.

4. Do the nomination and proposed research program merit NTP evaluation and if so, what priority (low, moderate, or high) should it be given?

High priority for NTP evaluation.

- Large knowledge gaps exist regarding the inhalation toxicity of ABF, diacetyl, and acetoin and the products are used in numerous occupational settings.
- Bronchiolitis obliterans is a serious lung disease with high morbidity and mortality and with lack of effective treatment. Preventing the disease by controlling exposure is the best strategy. The proposed studies would determine no effect levels for inhalation toxicity of ABF with regards to pulmonary toxicity and thus allow controls to be put into place to limit exposure of workers and minimize the likelihood that bronchiolitis obliterans develops.
- Mechanistic knowledge gained regarding ABF and bronchiolitis obliterans may be applicable in preventing pulmonary toxicity with other occupational exposures as well.
- Examination of 2 of the major components (diacetyl and acetoin) is warranted. If toxicity with these major components mimics the toxic pulmonary affects of ABF in the animal studies, then the toxicity of other minor components of the mixture will be a lower priority for study.

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